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13. ABSTRACT (Maximum 200 Words)
Folate receptors are overexpressed on a variety of human cancer cells, including breast cancer cells, but are restricted in normal tissues. Folate-conjugated radiopharmaceuticals have shown specificity for folate-receptor-bearing cells and promise in cancer imaging in animal models. We proposed to explore the coupling to folate of a series of MR contrast agents whereby ligands for complexation of paramagnetic metal ions, such as iron(III) and gadolinium(III) would be attached to folate and hence targeted to cancer cells. Folate-coupled ligands for these paramagnetic ions proved to be insufficiently soluble in aqueous media for separation, purification and utilization at the concentrations required for imaging purposes. In order to increase water solubility, poly(ethylene glycol) spacer units were incorporated into the conjugates and it was found that water soluble species could be obtained that incorporated folate bound via the spacer to fluorinated organics. These species were shown through imaging studies with phantoms to exhibit promise as ultra contrast agents and hence future work will focus on examining the uptake of these agnets by cells that overexpress the folate receptor. To that end, methodology has been developed to examine the uptake of the conjugates and determine their affinities for the folate receptor in specific cell lines.

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Introduction:

Folic acid is a vitamin that enters cells through either carrier-mediated or receptor-mediated processes. The latter processes involve a high-affinity folate receptor that promotes endocytosis and folate-drug conjugates may be targeted to these receptors.[1]

We proposed to synthesize contrast agents for magnetic resonance (MR) imaging conjugated to folate through modification of the gamma-carboxylate and, at the time of the 2002 annual report, had found that the syntheses explored, which were based upon literature reports, generated coupled products that contained unacceptably high levels of impurities and which resisted purification. The samples were unsuitable for imaging studies or for uptake studies with cell lines.

Body:

The folate-conjugated MR contrast agents were further purified by a range of chromatographic methods only to discover that they were insufficiently water soluble to obtain aqueous solutions in high enough concentration to be useful in imaging.

In order to increase water solubility we sought to employ a pegylated spacer group in the coupling of the folic acid to the contrast media such that the hydrophilic poly(ethylene glycol) moiety would enhance compatibility with an aqueous medium. To that end we attached a PEG linker to folic acid as follows:

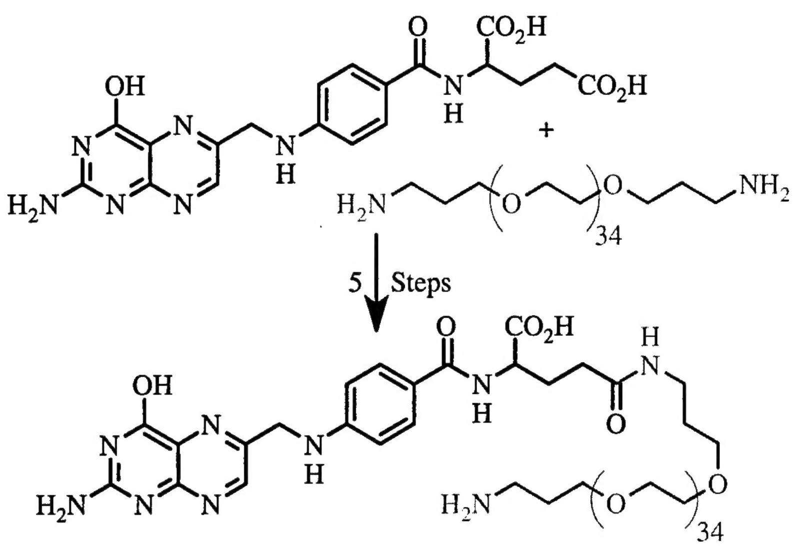


Figure 1. Attachment of PEG linker to folic acid.

Thus, folic acid was first dissolved in DMSO. Then, one molar equivalent of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) and four molar equivalents of N-hydroxysuccinimide were dissolved in DMSO and slowly added to the folic acid solution. This solution was then allowed to stir for four hours at room temperature. One molar equivalent of poly(ethylene glycol) bis-amine (MW ~ 3350 g/mol) was added to the solution and mixture was allowed to stir overnight (Figure 1). Acetone was added to the solution to precipitate a yellow solid. This solid was collected by centrifugation and washed four times with acetone. The solid was then dried and redissolved in DMSO.

In order to establish that contrast agents could be attached to the pegylated folate, a perfluorinated organic was employed, as follows. Five molar equivalents of pentadecafluorooctanoyl chloride and ten equivalents of pyridine were added to the DMSO solution of the pegylated folate and the solution was allowed to stir overnight at room temperature. The product was precipitated using acetone, collected via centrifugation and then washed four times with acetone before being dried under vacuum (Figure 2).

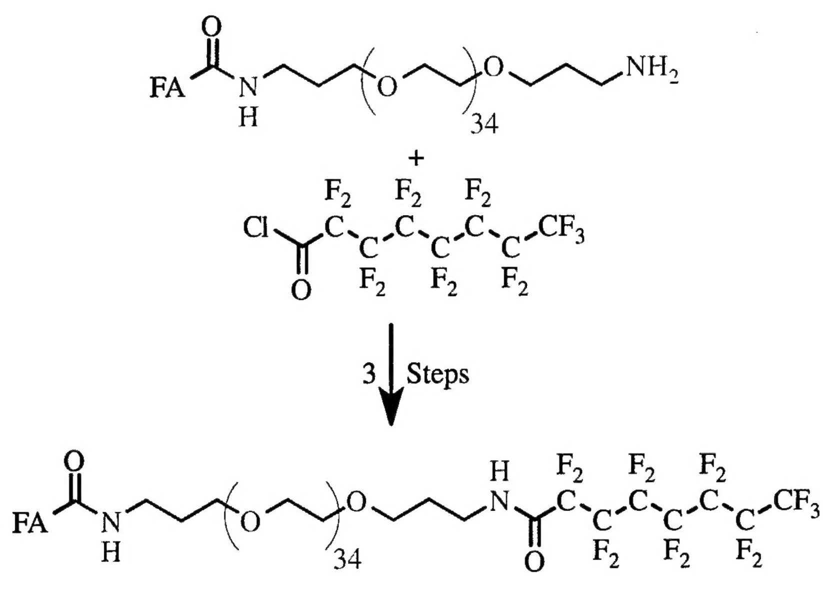
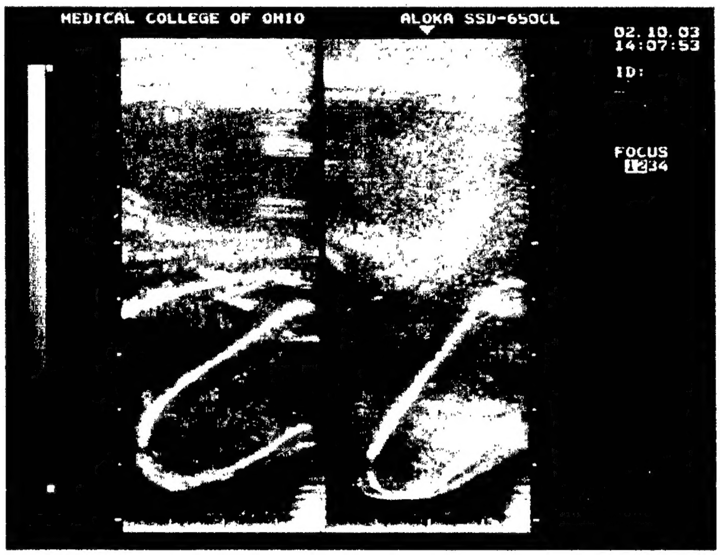


Figure 2. Attachment of perfluorocarbon chain to folic acid-PEG molecule.

The folate-conjugated perfluorocarbon was examined in a phantom imaging experiment as a potential ultrasound contrast agent. It was found to exhibit sufficient water solubility for this experiment, thus demonstrating that pegylation provides the solution to the problem of insolubility previously encountered. The experiment was performed as follows: The product was dissolved in water at a concentration of 20 mg/mL. This solution was poured into the finger of a latex glove and the finger was

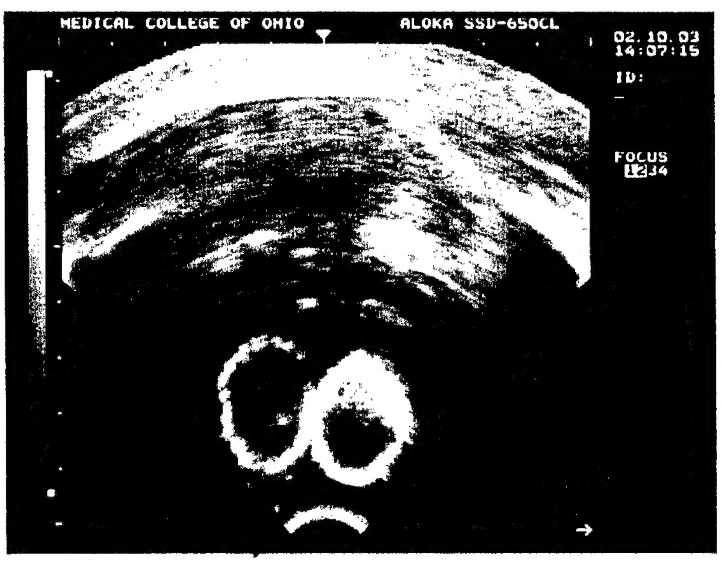
sealed. The adjacent finger was filled with water and similarly sealed. The glove was subsequently immersed in water and ultrasound images were obtained. Figure 3 shows the two fingers of the glove along the long axis, with one finger containing the contrast agent and the other containing water.



Water only Contrast Agent

Figure 3

Figure 4 shows a cross-sectional view of the fingers, again with one finger containing contrast agent and the other containing water.



Water only Contrast Agent

Figure 4

The ultrasound images obtained with the folate-contrast agent conjugate are promising. Additional work now needs to be done to test the folate-specificity of the conjugate and its utility in imaging tumors that over express folate receptors.[2] The methodology has been shown to be promising, however, and new classes of contrast agents may be anticipated. The work described here was performed in collaboration with colleagues at the Medical College of Ohio (Drs. Braun, Staren, Jankun, and Ratnam) and imaging experiments were undertaken with their equipment and performed by them.

Key Research Accomplishments:

The ultrasound images obtained with the folate-contrast agent conjugate are promising but uptake and specificity studies are yet to be performed in order to determine whether this agent will be a promising candidate for development in breast cancer imaging.

Reportable Outcomes:

There are no reportable outcomes at this time.

Conclusions:

Despite challenging problems of synthesis, purification and water solubility, progress has been made in developing folate-contrast agent conjugates that are suitable for uptake and specificity studies in order to determine whether such agents will be promising candidates for development in breast cancer imaging.

References:

[1] Gruner, B. and Weitman, S. (1999). "The Folate Receptor as a Potential Therapeutic Anticancer Target" Investigational New Drugs **16**: 205-219.

[2] Reddy, J, and Low, P. (1998). "Folate-mediated Targeting of Therapeutic and Imaging Agents to Cancers." Critical Reviews in Therapeutic Drug Carrier Systems **15(6)**: 587-627.